NOAA-NATIONAL OCEAN SERVICE

CENTER FOR COASTAL ENVIRONMENTAL HEALTH AND BIOMOLECULAR RESEARCH

FY02 SIGNIFICANT ACCOMPLISHMENTS

MARINE BIOTOXINS PROGRAM

cDNA LIBRARY PROVIDES MOLECULAR TOOLS TO UNDERSTAND HAB FORMATION

Understanding the mechanisms that control the growth and toxicity of dinoflagellates has long been hampered by our lack of insight into their molecular biology, stemming from the lack of molecular tools needed for such investigations. Development and screening of a cDNA library containing expressed gene sequences from the Florida red tide dinoflagellate, *Karenia brevis,* was therefore initiated this year to provide some of these tools. This project has yielded novel insights into the intracellular signaling pathways, cell cycle control, and stress response mechanisms present in this dinoflagellate species. To date, 1150 *K. brevis* expressed sequence tags (ESTs) have been sequenced. Of these, 36% have high homology to known genes in the GenBank database. Using these sequence data, we have developed probes for known cell cycle regulatory proteins to study the mechanisms controlling the growth phase of bloom formation and for stress proteins involved in adaptation/survival of *K. brevis* cells as they are exposed to changing water column conditions. Understanding cellular regulation is a prerequisite to developing truly predictive models or species-specific control strategies.

DEVELOPMENT OF A MOLECULAR PROBE FOR *KARENIA BREVIS*

The brevetoxin producing red tide dinoflagellate, *Karenia brevis*, is responsible for closure of shellfish harvesting as well as marine faunal mortalities throughout the Gulf of Mexico and along Florida's Atlantic coast. The serious economic impacts of this harmful algal bloom species have led to the establishment of rigorous monitoring programs for early detection of *K. brevis* and also of research programs aimed at modeling and ultimately forecasting bloom events. For both monitoring and research applications, enumeration of *K. brevis* cells by conventional microscopy is required but is time-consuming and can be problematic when morphologically similar species occur in the same water sample. We have developed a species-specific, fluorescently labeled probe targeting *K. brevis* ribosomal RNA that "lights up" *K. brevis* cells under the epifluorescence microscope and clearly distinguishes them from cells of co-occurring species. Application of this molecular probe will result in enhanced speed and accuracy of microscope-based counting of *K. brevis* cells. Future use in conjunction with advanced instrumentation such as flow cytometers and in-water sensors will establish this probe as a valuable tool facilitating the automated detection of *K. brevis* for a variety monitoring and research applications.

IDENTIFICATION OF AN ALGICIDAL COMPOUND FROM THE CYANOBACTERIA, *TRICHODESMIUM*

Blooms of the brevetoxin producing dinoflagellate, *Karenia brevis,* occurring in the eastern Gulf of Mexico are frequently preceded by blooms of the cyanobacterium, *Trichodesmium erythraeum*. However, in the western Gulf of Mexico, populations of the closely related *T. theibautii* appear to inhibit *K. brevis* bloom formation. Cell extracts obtained from these two *Trichodesmium* species were tested for growth effects against a diverse array of algal groups, including diatoms, dinoflagellates, green algae, and coccolithophores. Algicidal (i.e., algae killing) activity was observed for extracts of *T. theibautii*, while this activity was absent from *T. erythraeum* extracts. Our current research is aimed at determining the chemical structure of this algicidal compound and developing detection methods for use with field samples. Since the presence of *Trichodesmium* and its algicidal compound may regulate initiation of certain *K.*

brevis blooms, our ability measure levels of the algicide in natural populations may aid in assessing the potential for bloom formation.

TOXIN PURIFICATION FROM *PFIESTERIA* GROWN IN THE ABSENCE OF FISH

A new process was developed for producing *Pfiesteria* toxin(s) from cultures grown in the absence of fish. Prior to this development, toxin production by this dinoflagellate appeared to require the presence of fish within the culture. This situation complicated purification methods due to the presence of fish oils, proteins, byproducts, and other non-dinoflagellate debris, which clogged chromatography columns and required additional separation steps in order to obtain clean active samples. The longer time required by the more extensive purification protocol for extracts from fish-cultured algae resulted in unacceptable losses of toxin activity likely due to chemical decomposition. Our new method of culturing *Pfiesteria piscicida* without fish and using *Rhodomonas* sp. as the sole food source has yielded acceptable levels of toxin production and provided cleaner extracts that can be purified with a very rapid three-step procedure. This process has provided NOS scientists with a routine method to produce and maintain enough toxin(s) for structural analysis via 13C, 1H NMR as well as mass spectrometry.

DISCOVERY OF PSP TOXINS IN NORTH ATLANTIC RIGHT WHALES

Intensive study of the western North Atlantic right whale (*Eubalaena glacialis*) population over the past 20 years has yielded evidence of reproductive dysfunction in this highly endangered cetacean species. Among the factors identified as potentially contributing to this phenomenon, exposure to marine algal toxins has received little consideration. We recently initiated a study to investigate the possible occurrence of paralytic shellfish poisoning (PSP) toxins in *E. glacialis* and in the zooplankton assemblage comprising the majority of its diet. Samples of *E. glacialis* fecal material from at least ten different animals obtained during Aug./Sept. 2001 from the Bay of Fundy, Canada, tested positive for PSP toxins by both receptor binding assay and by HPLC analysis. Zooplankton samples collected during the same time period were also shown to contain similar levels of PSP toxins by weight using both methods. Additional data revealed the presence of toxic *Alexandrium* cells immediately before and during the sampling period. These findings provide the first compelling evidence for the occurrence of PSP toxins in *E. glacialis*, suggesting that further studies are warranted to examine the trophic transfer of these biotoxins via zooplankton vectors and their possible effects on the reproductive success of this endangered species.

IDENTIFICATION OF DOMOIC ACID AS A CAUSATIVE AGENT IN MASS MORTALITIES OF MARINE MAMMALS ON THE CALIFORNIA COAST

Extensive mortalities of marine mammals along the California coast occurred this year from February through June 2002. The event began with the mysterious deaths of dolphins along the California coast from San Lius Obispo to Orange County. Analysis of dolphin urine by the Marine Biotoxins Program's Analytical Response Team (ART) revealed high concentrations of the algal toxin, domoic acid (DA). Blooms of the DA-producing diatom, *Pseudo-nitzschia australis,* were subsequently found to be the source of toxicity and these blooms continued intermittently along the California coast through June, resulting in the deaths of large numbers of dolphins, sea lions, sea otters, and whales. Over 100 samples were analyzed by the ART to confirm the involvement of DA in mortalities of novel marine species and provide insight into trophic transfer of DA to dolphins and placental transfer of the toxin in gestating female sea lions. DA was previously identified by this Program as the causative agent in the mass mortality of California sea lions in the Monterey Bay area in 1998 and of California sea lions and sea otters in 2000. This is the first year in which multiple species of marine mammal were severely impacted from San Francisco south to Los Angeles along the central California coast, as well as the Baha, California coast and into Mexico.

TRANSFER OF RECEPTOR ASSAY TECHNOLGY TO SW AFRICAN COUNTRIES INITIATED

The southwest African countries of South Africa, Namibia, and Angola have either historical or recently emerging problems with one or more groups of marine algal toxins. These countries have requested assistance through the U.N. International Atomic Energy Agency (IAEA) in establishing capabilities for receptor assay-based detection of

algal toxins in seafood products. A project planning meeting was held at IAEA Headquarters in Vienna, Austria to develop a regional technical cooperation proposal for the transfer of the Marine Biotoxins Program's receptor assay technology to each of these three African countries. This project will be modeled after an ongoing IAEA-sponsored program in SE Asia, with the African end-users visiting the CCEHBR laboratory next year for training and returning to their home institutions to begin conducting the assays. An inter-calibration study coordinated through our Program will follow, and then receptor assays will be implemented as a component of their respective toxin monitoring programs, which are either well-established (S. Africa) or currently being developed. Acquisition of receptor-based technology will be of immediate benefit to each of our African partners, given their rapidly growing fishery and aquaculture industries along with the accompanying demands for biotoxin testing of products for export to world markets.

NEW RADIOIMMUNOASSAY PROVIDES SENSITIVITY REQUIRED TO BIOMONITOR BLOOD BREVETOXIN

The need for definitive toxin identification associated with harmful algal blooms is increasing. However, the relationship between exposure to toxins in the environment and adverse effects is poorly defined. Biomonitoring of toxins in human or animal tissue using blood collection cards provides an efficient means to identify toxins in living animals in order to assess exposure. Collaborative research between CCEHBR and AgResearch Ltd. (New Zealand) has resulted in a new radioimmunoassay for brevetoxins. The radioimmunoassay provided sensitive detection with minimal interference of residual matrix following extraction of blood samples from the cards. The method has been tested in laboratory mice after acute, long term, and low dose exposure to brevetoxins and was determined to detect blood brevetoxin at doses ten times below the lowest observable effect dose and at times up to two days after exposure. Analysis of toxins extracted from blood collection cards using this radioimmunoassay will permit biomonitoring of blood brevetoxin during HAB events and can be anticipated to provide early indications of toxic events and to enhance our ability to predict the toxic consequences of red tides in protected species.

DIETARY CHOLESTYRAMINE PREVENTS BREVETOXIN SYMPTOMS

There is a need to develop prophylactic treatment to alleviate the adverse effects of dietary exposure to marine algal toxins. Dietary treatment with cholestyramine is one possible means of either binding or disrupting enterohepatic circulation of toxins and thereby mitigating toxic symptoms. While cholestyramine has been used successfully to block the toxic effects of mycotoxins and other toxicants, there is little supportive experimental evidence with algal toxins. Work in collaboration with the U.S. EPA laboratory in Research Triangle Park, incorporated cholestyramine into the diet of laboratory mice, which were then exposed orally to brevetoxin. A 5% (w/w) diet of cholestyramine for one week prior to toxin exposure eliminated the characteristic hypothermic response to a nonlethal dose of brevetoxin. Mice fed the cholestyramine diet displayed no observable symptoms to oral brevetoxin exposure, even when given a higher dose of brevetoxin that caused 50% lethality in one hour in the mice fed the control diet. These exciting results provide the first experimental data that support the effectiveness of this commonly used cholesterol lowering drug to mitigate the adverse of effects of brevetoxin.

CONFIRMATION OF PSP IN THE INDIAN RIVER LAGOON: A NEW PUBLIC HEALTH ISSUE IN FLORIDA

Between 1 January – 25 April 2002, 14 pufferfish poisoning incidents in Florida were reported from the Indian River Lagoon. (IRL), Florida. In collaboration with the Florida Marine Research Institute (FMRI), the U.S. FDA, and NRC Canada, we have confirmed the presence of saxitoxins (STX) in pufferfish in the IRL. This is the first toxic event in Florida waters in which STX has been identified. Concentrations of up to 6238 µg STX eq/100 g were detected in skin, mucus, muscle, and viscera of southern pufferfish, with highest levels in the skin and mucus. STX was also confirmed in conch (*Melongena corona*) and cockle (*Americardia media*) from the IRL, with traces detected in hard clams (*Mercenaria* spp.). Both unialgal cultures of the dinoflagellate *Pyrodinium bahamense* and natural bloom samples (> 3 million cells/L) obtained during fish kills in the IRL tested positive for STX. *Pyrodinium bahamense* var. *bahamense*, the variety found in Florida, has never before been reported to be toxic. Current studies at FMRI are aimed at confirming the origin of the toxic blooms present in the IRL. Recently, the

northern IRL has experienced a number of unusual events, including dolphin, manatee, fish, and horseshoe crab mortalities, increased tumor incidence in hard clams, "spicy clams," and reduced natural recruitment and hatchery losses of hard clams. To what extent these events are linked to the emerging issue of toxic *P. bahamense* blooms remains undetermined. Public health risks associated with PSP have resulted in the implementation of new management strategies by the state of Florida, which previously has had to regulate shellfish harvests only for brevetoxins.

VOLUNTEERS MONITOR HARMFUL PHYTOPLANKTON ALONG SOUTH CAROLINA COAST

The South Carolina Phytoplankton Monitoring Network (SCPMN) began its second year of existence with over 34 groups monitoring state coastal waters for potentially harmful algal species. A total of over 50 sampling sites from all coastal counties of South Carolina are monitored each week. Volunteer groups are composed of both middle and high school students, state park personnel, and citizen environmental groups. This NOAA sponsored community program serves to increase the awareness of constituent groups about the many issues related to harmful algae and directly involves volunteers in coastal stewardship. In the SCPMN's first year of existence, volunteers observed three potentially toxic algae, including *Pseudo-nitzschia*, *Dinophysis*, and *Prorocentrum lima*. Observation and identification of phytoplankton along the South Carolina coast will be useful in developing a species list and record of distribution, as well as alerting NOAA scientists to the presence of potentially harmful species at the many sampling sites.